III.5 — STABLE ISOTOPE MEASUREMENTS OF DISSOLVED INORGANIC CARBON AND SOIL GASES AT TWO BIOREMEDIATION SITES

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INTRODUCTION

Contamination of soil, sediment, and waters by benzene, toluene, ethylbenzene, xylene (BTEX) and polycyclic aromatic hydrocarbons (PAHs) is one of the world's largest environmental problems. Given the scope and magnitude of these contamination problems, in situ bioremediation often represents the only viable remedial solution from a practical applications perspective. A primary problem in monitoring the efficacy of bioremediation is the difficulty in measuring the degradation rates of individual compounds and relating the disappearance of compounds to bacterial activity. Stable isotope analyses are being used increasingly to study pollution [10] and may be a powerful tool for proving that microorganisms are affecting the clean-up of a contaminated site.

By measuring the stable carbon isotope ($\delta^{13}C$) values of constituents of interest (COI), background-indigenous carbon sources, bacterial biomass, and respired CO_2, the flux of COI through bacterial biomass and into respiration products can be verified. The use of $\delta^{13}C$ for tracing indigenous carbon sources through microbial degradation has been successful in aqueous environments [2,3,4]. In bioremediation settings, if bacteria are consuming pollutant compounds, their biomass should have a $\delta^{13}C$ signal similar to that of the pollutants (see Pelz et al., Chapter II.5). Furthermore, the CO_2 they respire should also have the same carbon isotopic signal (see Trust et al., Chapter II.1). Depending on where degradation is occurring, respired CO_2 will either diffuse through the vadose zone or dissolve in the groundwater and increase the dissolved inorganic carbon (DIC = $CO_2 + HCO_3^- + CO_3^{2-}$) concentration.

The $\delta^{13}C$ of soil CO_2 is generally 4‰ to 5‰ more positive than the associated carbon source (e.g., vegetation, soils, contaminant) owing to diffusion and mixing with atmospheric CO_2 (-8‰; 1). In turn, groundwater DIC has carbon isotope ratios that are intermediate between soil CO_2 and those of carbonates, which are typically ^{13}C-enriched (-12‰ to $+2$‰; [1]). Thus, degradation of contaminant may alter the isotopic ratio of both DIC and soil CO_2 if (1) the COI has a unique $\delta^{13}C$ value, or (2) the production of CO_2 is enhanced significantly and dilution of signal by carbonate dissolution and air invasion is diminished. Since petroleum is depleted in ^{13}C (-32‰ to -24‰; [14]) compared to carbonates and/or air, degradation of petroleum-derived contaminants should lead to more negative $\delta^{13}C$ in DIC or soil CO_2.

The objective of this study was to determine if measurements of stable carbon isotopes could be used as in situ tracers of the assimilation and respiration of COI by bacteria at a bioremediated site. Our study areas included a BTEX-impacted site (Naval Construction Battalion Command, Port Hueneme, California) and a creosote-impacted site (Cabot Carbon/Kopper's Industries Superfund Site, Gainesville, Florida). Both sites had an in situ bioremediation system based on vertical groundwater circulation well (GCW)

technology (see Mueller et al., Chapters I.1 and I.2). Soil samples were taken initially, and isotopic determinations of soil organic matter, humic acids, and adsorbed COI were performed. Over a 1-year period, we sampled 20 wells at Port Hueneme and 12 wells at Gainesville. The $\delta^{13}C$ was analyzed in groundwater DIC and particulates and in soil gas CO_2. In addition, we measured salinity, pH, temperature, and DIC concentration. Below, we compare the isotopic data in impacted and "background" sites for winter, spring, and summer 1995. In turn, we assessed whether the GCW had an effect on the isotope ratios by comparing wells near to the GCW with those at greater distance. Although the isotopic ratio of the pollutants (BTEX and PAH at the two study sites) were quite similar to those of the soil organic carbon and the associated humic acids, there were distinct isotopic differences among soil gas CO_2 sampled in wells in close proximity to the COI when compared with those sampled at background sites. Thus, it appeared that there was enhanced CO_2 production in the impacted sites, which had an isotopic ratio that was distinct from that forming the majority of the CO_2 in the background area.

STUDY SITES

Port Hueneme

One study was conducted at the Naval Construction Battalion Command, which serves as the U.S. Navy's National Hydrocarbon Test Site. About 4,000 gal of regular gasoline and 6,800 gal of premium unleaded gasoline leaked from underground delivery lines at this site between September 1984 and March 1985. Details of the geology, hydrology, and plume delineation are found in Mueller et al. (Chapter I.1).

Gainesville

The Cabot Carbon/Kopper's Superfund Site served as the second field site. Since the early 1900s, a combination of pine-tar, charcoal-generation, and wood-treating operations took place at the site. Alternatively, creosote, pentachlorophenol, and chromated copper arsenate were used to preserve wood utility poles and timbers. Wood-treating operations continue in a portion of the site, but only chromated copper arsenate is being used presently. A more explicit description of the site and extent of releases associated with the plant operations is found in Mueller et al. (Chapter I.2).

Contaminated and Background Wells

In the data analyses below, we have separated the wells into different areas (Table 1). Background wells were located in areas with significantly lower COI concentration. Three rings of impacted wells (four per ring) were located around the GCW system. We separated these wells into inner (ring next to GCW) and outer (two outside rings) wells. Although COI concentrations were similar for inner and outer wells, this division was designed to test for the influence of the GCW. Maps of COI plumes and well placement are found in Mueller et al. (Chapters I.1 and I.2).

MATERIALS AND METHODS

Field Sampling

A 4-L bottle was filled slowly from the bottom with a peristaltic pump to minimize atmospheric mixing. Sample aliquots for DIC analysis were collected by drawing 50 mL of water from the bottom of the 4-L bottle using a syringe and transfer line and overfilling a 30-mL Quorpak bottle. The sample was fixed with 250 μL of 2% $HgCl_2$ to retard biological activity and stored headspace-free at 4° C. Samples for DIC isotope ratios were collected similarly. In turn, groundwater particulate samples were prepared by filtering groundwater through a combusted 47-mm glass-fiber/filter (GF/F) until the filter was discolored. The filter was then frozen. Samples were taken in duplicate for DIC concentration and isotopic ratio measurements and for groundwater particulates.

Table 1 — Concentrations at Background and Impacted Wells at the Port Hueneme, (BTEX impacted; January 1995) and Gainesville (PAH impacted; February 1995) sites. (Background wells at Port Hueneme included #13,14,19,20. Background well sites at Gainesville were chosen because PAHs were below detection. Impacted wells were divided into inner (#3,6,7,10) and outer rings (#1,2,4,5,8,9,11,12). Refer to Mueller et al. (Chapters I.1 and I.2) for maps of well locations.)

Site	Background Mean (ppm)[1]	Inner Mean (ppm)[1]	Outer Mean (ppm)[1]
Port Hueneme [BTEX]	0.002 ± 0.002	75.3 ± 29.1	94.4 ± 21.9
Gainesville [PAH]	Below detection	6.0 ± 2.4	4.9 ± 5.6

[1]see Kelley at al. (Chapter III.3) and Trust et al. (Chapter III.4)

Carbon-dioxide gas samples were collected by first drawing and discarding 180 mL of gas from the well. A sample was then drawn using a syringe with an on/off valve. Eight mL of sample were injected into an evacuated 6-mL Pierce serum vial with a red rubber stopper. Overpressurization ensured that no outside air contaminated the sample. Methane samples were drawn in a similar fashion and injected into 15-mL Pierce vials filled with water and held upside down in a bucket, allowing the sample gas to displace the water. The methane sample bottles were sealed with blue butyl rubber stoppers. Apiezon grease was applied to seal both types of samples.

Soil samples were collected using a piston core. Soil aliquots for humic analysis were collected in 1-L jars and frozen.

LABORATORY ANALYSES

The isotopic ratio of CO_2 was determined by gas chromatography/isotope ratio mass spectrometry (GC/IRMS) [13]. Cryogenic focusing was typically used for sample concentrations below 1%. Prior to introduction into the IRMS, the CO_2 was separated from other gases (e.g., N_2, Ar, CO, CH_4) with a Carboxen 1006 Plot column (30 m, 0.53 mm i.d., Supelco, Bellefonte, Pennsylvania). Details are found Salata et al. (Chapter II.4). The precision for duplicate samples taken in the field averaged ±0.3‰.

The isotope ratio of DIC was measured by headspace analysis (see Salata et al., Chapter II.3). Samples were introduced into an evacuated 2-mL vial that contained 100 μL of concentrated phosphoric acid. Isotopic equilibrium between solution and headspace was reached after 18 h. At this time, an automated headspace analyzer that was connected to the GC/IRMS was used to introduce the sample into the GC. The isotopic ratio of the CO_2 gas was measured, as described. Precision for samples taken in the field averaged ±0.3‰.

In preparation for isotopic analyses, soil samples were placed in 10% acid to remove carbonates. Acidified soils were retained on a 47-mm GF/F and washed until neutralized. After drying (60° C, 24 h), the soil was scrapped off the filter. Humic acids were extracted according to Ertel and Hedges [6].

Filters with groundwater particulates were dried at 60° C, for 24 h and placed in a dessicator with acid fumes for 12 h to remove inorganic carbon. In turn, the filters were placed in the oven (60° C, 24 h) again to ensure removal of residual water and acid.

Organic carbon in soils, humic acids, and groundwater particulates were converted to CO_2 by a modified Dumas combustion [9]. The CO_2 gas was separated from other gases by cryogenic distillation and transferred to sample bulbs. The precision for these sets of samples was $\pm 0.2‰$.

All carbon isotope analyses were performed on a Finnigan MAT 252 GC/IRMS. We present the results in standard notation:

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{std}} - 1 \right] \times 1000, \quad (1)$$

where $(^{13}C/^{12}C)_{sample}$ is the isotope ratio in the sample, and $(^{13}C/^{12}C)_{std}$ is the isotope ratio in the standard, Pee Dee Belemnite, that by definition has a $\delta^{13}C$ of 0.0. The working standard was a tank gas (Oztech Trading Corp.), which has a $\delta^{13}C$ of -37.8 vs Pee Dee Belemnite. Precision of the various isotopic measurements was described earlier.

Both BTEX and PAH isotope ratios were measured by GC/IRMS at the Environmental Protection Agency's Environmental Research Laboratory, Gulf Breeze, Florida, on a Finnigan Delta-S equipped with a GC interface. Details of the methodologies are found in Kelley et al. (Chapter II.2).

The DIC concentration was determined coulometrically with a UIC model 5011 coulometer. A 10-mL volume of the preserved sample was placed in an apparatus that stripped all evolved CO_2 following acidification. The displaced CO_2 was transferred to the coulometer by a stream of ultrahigh-purity nitrogen, where a pH-dependent reaction altered the transmittance in a collection cell. The concentration was determined by the amount of electrode-generated hydroxide ion required to return the transmittance to its baseline level [7]. The typical precision for this type of measurement was $\pm 0.5\%$.

RESULTS AND DISCUSSION

Sources for Microbial Degradation

The $\delta^{13}C$ of indigenous organic matter (soil organic matter and humic acids) was not significantly different from COI at either the Port Hueneme or Gainesville sites (Table 2). Values at these sites were in the range of $-25‰$ to $-23‰$. In contrast, the $\delta^{13}C$ of groundwater particulates were comparatively ^{13}C-depleted, particularly at Port Hueneme (Fig. 1) where values were more variable and negative at depth. Since the majority of organic matter in both of these environments was associated with soils and COI—not with the groundwater particulates, we hypothesized that ^{13}C-depletion in groundwater particulates reflected the presence of chemosynthetic bacteria that are relatively ^{13}C-depleted [8]. Thus we contend that the groundwater particulates did not represent another large source of organic matter with a different $\delta^{13}C$ but instead was influenced by the degradation of soil and/or COI organic matter. For example, at Port Hueneme, the ^{13}C-depleted groundwater particulates in the deep wells would be consistent with anaerobic processes, such as sulfate or CO_2 reduction. In summary, the isotopic analyses of organic sources suggest that, in the absence of another unidentified but significant organic source, the $\delta^{13}C$ of soil CO_2 at Port Hueneme and Gainesville should be in the range of -25 to -8. The former is the value of the major organic sources and the latter is the value for atmospheric CO_2. Although we do not have soil carbonate values to report, similar ranges were anticipated for DIC at these sites.

Table 2 — Mean Stable Carbon Isotope Ratios ($\delta^{13}C$) of Soil Organic Matter, Humic Acids, Groundwater Particulates, and COI at the Port Hueneme (BTEX impacted; January 1995) and Gainesville (PAH impacted; February 1995) Sites. (Values for COI are weighted averages of the individual compounds.)

	Port Hueneme $\delta^{13}C$(‰)	Gainesville $\delta^{13}C$(‰)
Soil Organic Matter	−23.3±0.2	−23.8±0.7
Soil Humic Acid	−23.7±0.5	−23.0±0.5
Soil Contaminant	−23.9[1]	−24.4[2]
Groundwater Particulates Shallow	−29.8±2.0[3]	−26.4±2.8
Groundwater Particulates Deep	−33.2±2.9[3]	−26.8±1.9

[1]BTEX, see Kelley et al. (Chapter III.3)
[2]PAHs; see Trust et al. (Chapter III.4)
[3]Data taken in March 1995

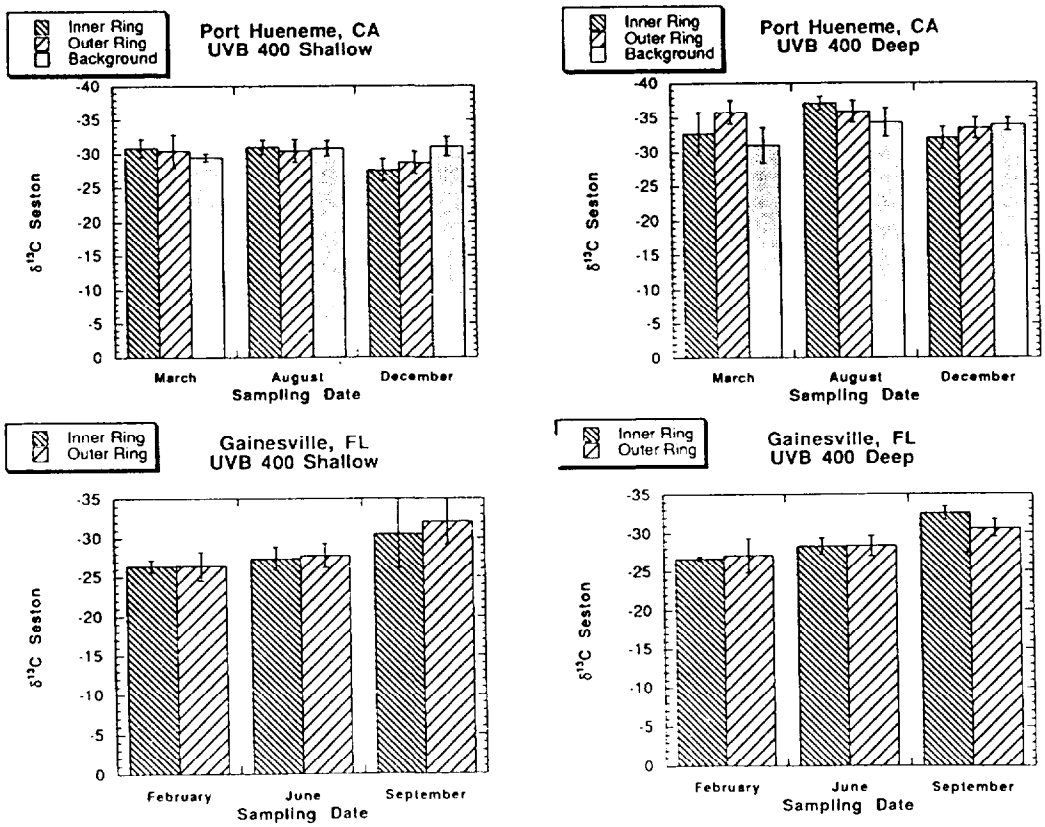

Fig. 1 — Stable carbon isotope ratio ($\delta^{13}C \pm S.D.$) of seston samples in Port Hueneme and Gainesville

Soil CO_2 and DIC at Port Hueneme

During January 1995, there was an unexpectedly large rainfall in the Port Hueneme area. Meanwhile, soil CO_2 isotope ratios at the background wells, which only had ppb levels of BTEX constituents (Table 1), were significantly depleted in ^{13}C (Fig. 2) compared with BTEX, soil organic matter, and humic acids (Table 2). Since the majority of organic matter is associated with soil material or BTEX, the more negative values in soil CO_2 (also observed in groundwater particulates) implied that another source of organic matter was contributing to soil CO_2. A possibility is methane oxidation, which produces ^{13}C-depleted bacteria (possibly associated with groundwater particulates) and respired CO_2, owing to the relatively negative value of methane (−80‰ to −30‰) and the isotope effect associated with the reaction [5]. High concentrations of methane were, in fact, measured in the soil gas in January 1995, whose isotope ratio for a majority of the samples decreased as that of the CO_2 increased (Fig. 3). The data shown in Fig. 3 were indicative of methane oxidation. Based on the unusual weather conditions (100-yr rains) at this time, however, we will not continue to use the January 1995 data for further temporal or spatial comparisons.

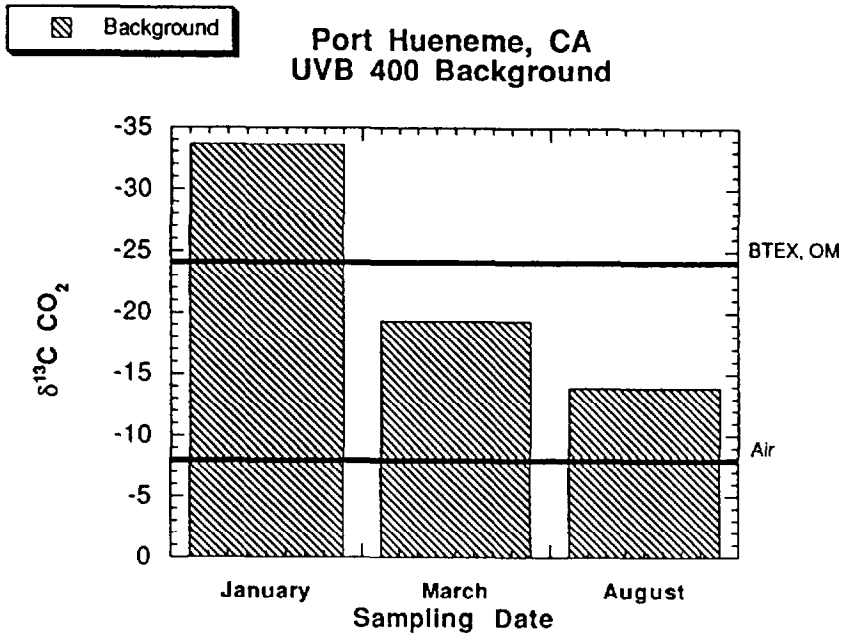

Fig. 2 — Stable carbon isotope ratio ($\delta^{13}C \pm$S.D.) of soil CO_2 sampled in January, March, and August 1995 at Port Hueneme. Data taken at background wells (#13,14,19, 20).

Soil gas $\delta^{13}C$ increased in March and August 1995 at the background wells (Fig. 2). Values were consistent with previous observations in unimpacted soils [1], intermediate between soil organic matter and that of atmospheric CO_2. The decrease between March and August 1995, if significant, could be explained by reduced degradation activity, leading to less CO_2 production and more dilution of the isotopic signal by atmospheric CO_2. An alternative explanation is that the soil became more desiccated, allowing greater infiltration by air.

Owing to the high DIC concentrations in the groundwater, changes in its isotopic ratio were more buffered when compared with soil CO_2. This was evident in the data from deep wells (Fig. 4(a)), which displayed little temporal or spatial variation. In contrast, the $\delta^{13}C$ of DIC in shallow wells (Fig. 4(b))

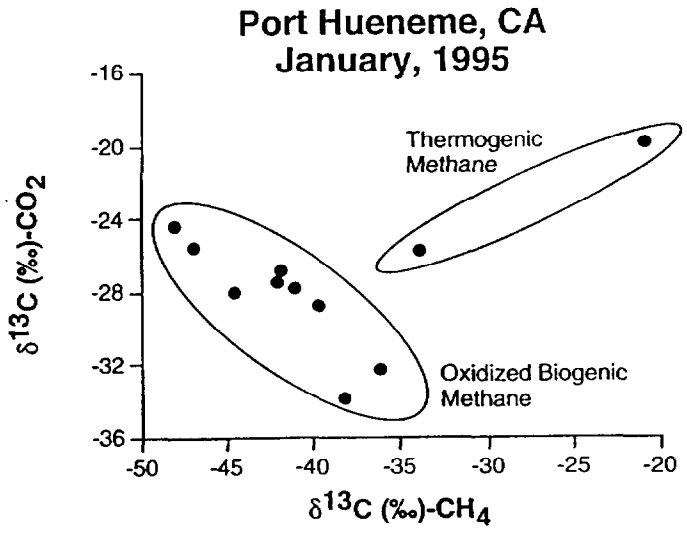

Fig. 3 — Stable carbon isotope ratio ($\delta^{13}C$) of soil CO_2 vs methane

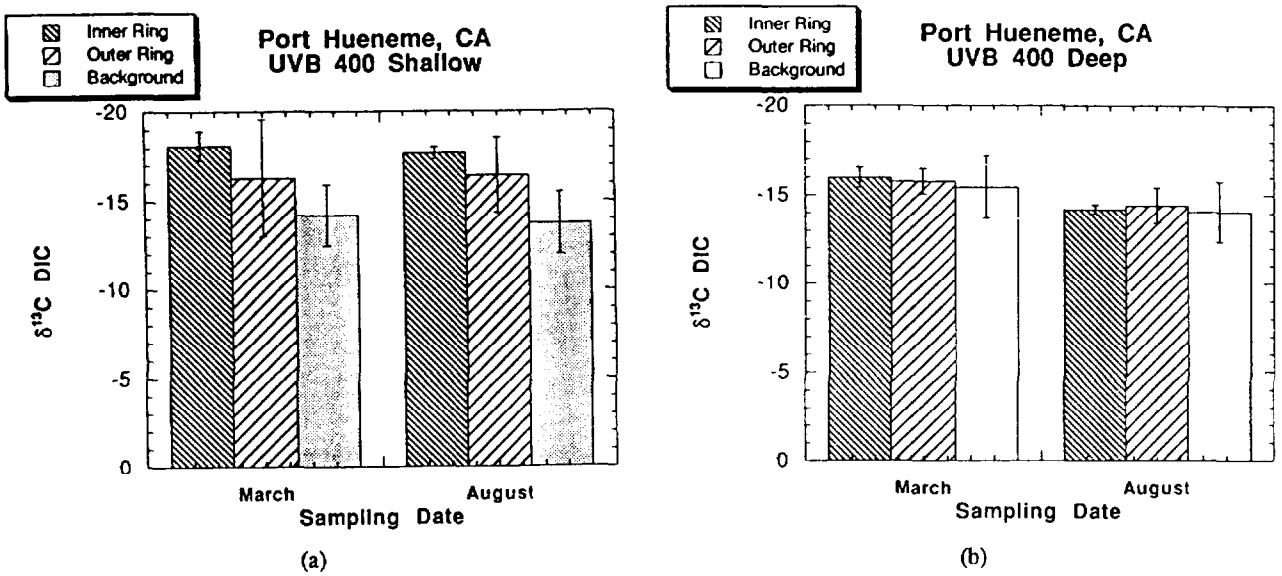

Fig. 4 — Stable carbon isotope ratio ($\delta^{13}C \pm$S.D.) of DIC sampled in March and August 1995 at Port Hueneme. Data taken from (a) shallow and (b) deep wells are separated into inner, outer, and background wells (Table 1).

were ^{13}C-depleted in the impacted wells. Moreover, with the exception of the background wells, the isotopic ratio of DIC was more negative in the shallow wells when compared with deep wells. The concentration of DIC was also much greater at the impacted shallow wells when compared with both background wells and deep wells (Figs. 5(a) and (b)). This clearly shows that there was a considerable input of CO_2 at shallow depths in the COI plume.

Considering the uniformity in the $\delta^{13}C$ of characterized organic sources, the spatial variations observed in isotope ratios of DIC (Figs. 4(a) and (b)) and soil CO_2 (Fig. 6) were explained either by an uncharacterized source of CO_2 or by greater inputs of respired CO_2 within the plume. In both March and August 1995, $\delta^{13}C$ values were more negative in the vicinity of the GCW. This observation was more

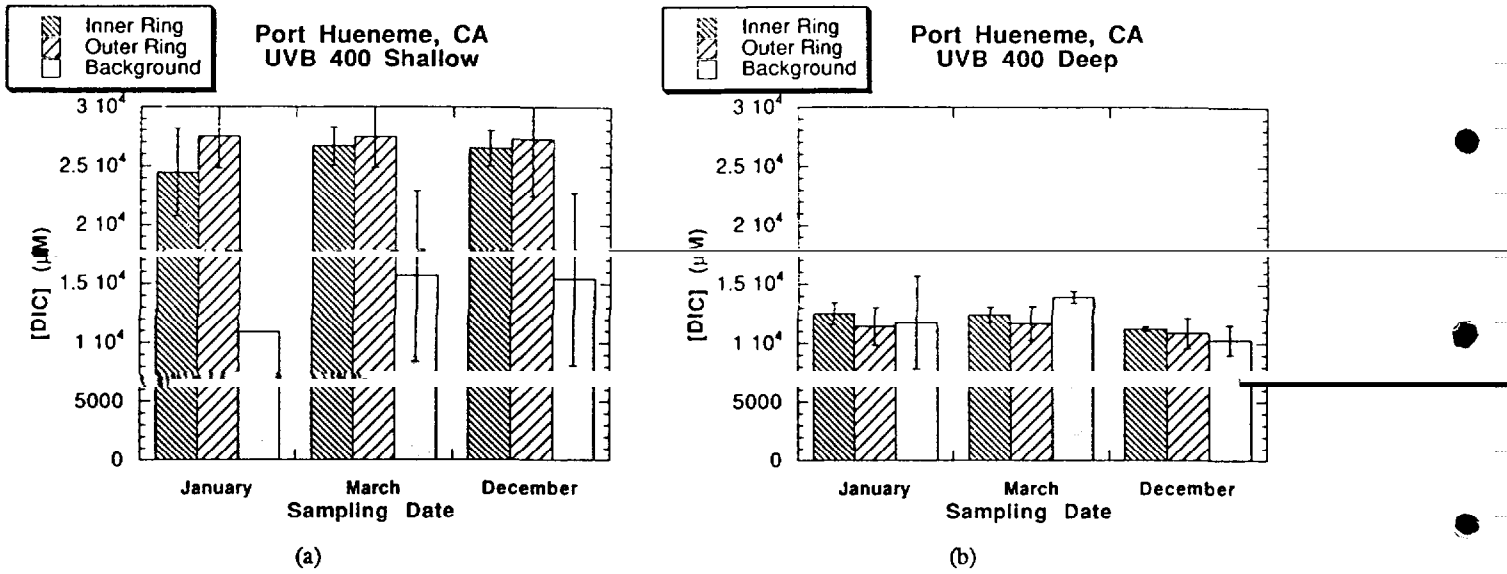

Fig. 5 — Concentration of DIC (μM$\pm$S.D.) sampled in January, March, and December 1995 at Port Hueneme. Data taken from (a) shallow and (b) deep wells are separated into inner, outer, and background wells (Table 1).

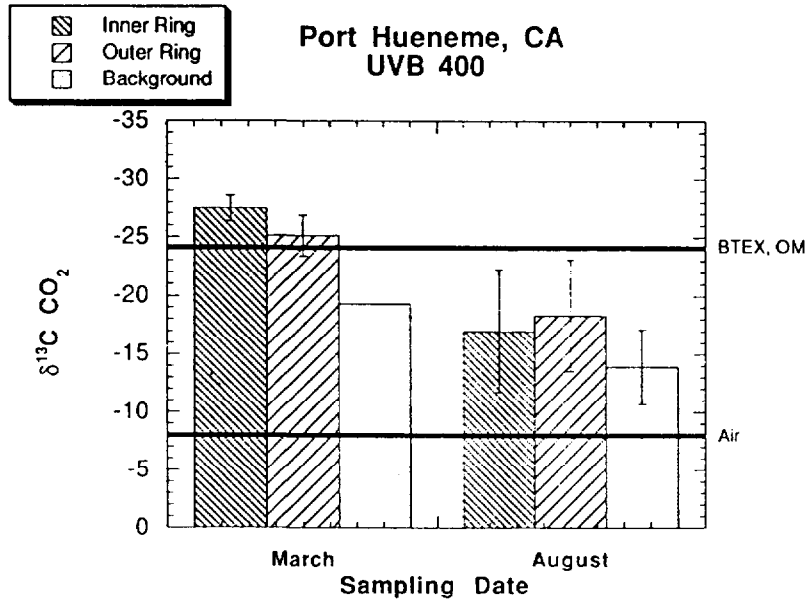

Fig. 6 — Stable carbon isotope ratio ($\delta^{13}C\pm$S.D.) of soil CO_2 sampled in March and August 1995 at Port Hueneme. Soil gas data are separated into inner, outer, and background wells (Table 1).

evident in March than in August 1995. To estimate the $\delta^{13}C$ of the respired CO_2, we applied a simple mass-balance calculation:

$$\delta^{13}C(DIC)_{shallow} = \frac{\delta^{13}C(DIC)_{deep} \cdot [DIC]_{deep} + \delta^{13}C(DIC)_{resp} \cdot [DIC]_{resp}}{[DIC]_{total}}, \quad (2)$$

or

$$\delta^{13}C(DIC)_{resp} = \frac{\delta^{13}C(DIC)_{shallow} \cdot [DIC]_{total} - \delta^{13}C(DIC)_{deep} \cdot [DIC]_{deep}}{[DIC]_{total} - [DIC]_{deep}}, \quad (3)$$

where resp designates the DIC produced by degradation in the shallow groundwater. The use of these equations was based on the assumption that the respired CO_2 added at shallow depths was equal to the difference between DIC concentrations at shallow depth in the COI plume and those at depth (Table 3). This assumption was supported by the uniformity among DIC concentration at background wells and at deep wells (see Figs. 5(a) and (b)). Based on equilibrium isotope calculations, the $\delta^{13}C$ of the aqueous CO_2 in inner- and outer-ring wells was similar to that of the soil CO_2 but was more negative than that of soil organic matter or COI (Table 1). The ^{13}C depletion observed in the soil CO_2 from contaminated wells implies another source of organic matter—most likely methane oxidation, as discussed earlier.

Soil CO_2 and DIC at Gainesville

In contrast with the Port Hueneme site, consistent spatial or temporal variations in the isotope ratios of soil CO_2 or DIC were not observed (data not shown). Concentrations of DIC were also much lower than at Port Hueneme and did not vary greatly between deep and shallow wells (Table 3). Equilibrium isotope calculations were also performed on the Gainesville data, but we were not able to separate out the $\delta^{13}C$ of added CO_2, owing to the uniformity in DIC concentrations between shallow and deep wells and the lack of background samples prior to the September 1995 sampling. The calculations with the total DIC data did show a strong similarity between the isotopic ratio of aqueous CO_2, soil CO_2 near the GCW, and soil CO_2 taken at a background well in September 1995 (-19.5 ± 0.2‰). Soil CO_2 gas, however, is typically 4‰ more positive that the prevailing organic sources [1]. In contrast, particularly next to the GCW during February, the $\delta^{13}C$ of soil CO_2 was almost equal to that of soil organic matter and COI (Table 2). The absence of isotopic discrimination usually observed in soil gas CO_2 with respect to organic sources can be explained by a large flux of CO_2 associated with degradation of the abundant COI. Under these circumstances, the 4‰ enrichment usually associated with diffusion and mixing with atmospheric CO_2 in impacted soils was not observed.

CONCLUSIONS

Unfortunately, the isotopic similarity between indigenous and COI organic matter at both sites did not provide ideal circumstances for validating the use of stable isotope as tracers of bioremediation. At Gainesville, $\delta^{13}C$ values of dissolved and soil CO_2 were essentially equivalent to those of soil and PAHs. The absence of isotopic discrimination between these pools suggests that the production of CO_2 was tightly linked to degradation of the available organic matter as predicted by the study of Trust et al. (see Chapter II.1). Furthermore, the flux of CO_2, which likely does not derive from abiotic processes, must have been sufficiently large to counter the ^{13}C-enriching effect of diffusion and mixing with atmospheric CO_2 reported in other environments [1]. The isotopic data from Gainesville, combined with the loss of parent compounds (see Mueller et al., Chapter I.2), provides strong evidence for a biological component to the removal of these COI.

Table 3 — Average concentration (μM ± S.D.) and Stable Carbon Isotope Ratios ($\delta^{13}C$ ± S.D) of DIC in Shallow and Deep Wells from Gainesville (PAH impacted). (The isotope ratio of aqueous CO_2 calculated according to isotopic equilibrium [15,12] is compared with that from the soil gas).

	[DIC]	$\delta^{13}C$(‰) DIC	$\delta^{13}C$(‰)[1] $(CO2)_{aq}$	$\delta^{13}C$(‰) $(CO2)_{gas}$
Pt. Hueneme March 1995 Shallow Inner Ring	24480± 3710	−18.2± 0.8	−27.8	−27.5± 1.1
Pt. Hueneme March 1995 Shallow Wells Outer Ring	27469± 2625	−16.3± 3.3	−25.1	−25.1± 1.7
Pt. Hueneme March 1995 Deep Wells	11607± 2080	−15.5± 1.0	N.A.	N.A.
Gainesville February 1995 Shallow	4564± 2534	−19.0± 2.4	−23.1	−23.3± 2.0
Gainesville February 1995 Deep	3433± 1353	−17.5± 2.5	N.A.	N.A.
Gainesville June 1995 Shallow	3826± 2627	−17.7± 1.8	−21.0	−19.9± 0.8
Gainesville June 1995 Deep	3478± 1197	−17.7± 1.4	N.A.	N.A.

[1]Only mean value shown. Equilibrium isotope calculations for Port Hueneme were based on the $\delta^{13}C$ of the added DIC (refer to Eq. (2)), whereas those for Gainesville were based on the $\delta^{13}C$ of total DIC.

At Port Hueneme, the $\delta^{13}C$ data are best explained by a process other than aerobic degradation of the soil organic matter and BTEX. For example, isotope ratios of dissolved and soil CO_2 measured in March 1995 were ^{13}C-depleted compared with the available organic matter. Thus, another source of organic matter must have contributed to the production of CO_2 that led to high concentrations of ^{13}C-depleted DIC observed in surface groundwater within the plume and to negative isotope ratios in the overlying soil CO_2. We submit that anaerobic processes were important to COI degradation at this site (see Morin et al., Chapter I.3), possibly methane production in the groundwater followed by oxidation in the vadose zone.

This preliminary view of the inorganic carbon isotope data, however, demonstrates the need for an additional tracer. Owing to the petroleum-based nature of the COI, the logical choice is to make ^{14}C-abundance measurements of CO_2 [11] and of the microbial biomass (see Salata et al., Chapter II.4). However, this approach, by nature of its greater analytical complexity and cost, should only be

undertaken after it has been determined that the stable isotope (e.g., ^{13}C) approach will not provide sufficient discrimination.

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